

5       What is claimed is:

1.   A method of assessing the effectiveness of protease antiretroviral therapy of an HIV-infected patient comprising:

- 10           (a) collecting a plasma sample from the HIV-infected patient;
- (b) evaluating whether the plasma sample contains nucleic acid encoding HIV protease having a mutation at codon 88; and
- 15           (c) determining increased susceptibility to amprenavir.

2.   The method of claim 1, wherein the mutation at codon 88 codes for a serine (S).

3.   The method of claim 1, wherein the HIV-infected patient is being treated with an antiretroviral agent.

4.   A method of assessing the effectiveness of protease antiretroviral therapy of an HIV-infected patient comprising:

- 25           (a) collecting a plasma sample from the HIV-infected patient;
- (b) evaluating whether the plasma sample contains nucleic acid encoding HIV protease having a mutation at codon 88 and additional mutations at codons 63 and/or 77 or a combination thereof; and
- 30           (c) determining decreased susceptibility to nelfinavir and indinavir and increased susceptibility to amprenavir.
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5.   The method of claim 4, wherein the mutation at codon 63 codes for a proline (P) or a glutamine (Q) and the mutation at codon 77 codes for an isoleucine (I).

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6. The method of claim 4, wherein the HIV-infected patient is being treated with an antiretroviral agent.

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7. A method of assessing the effectiveness of protease antiretroviral therapy of an HIV-infected patient comprising:

15

(a) collecting a plasma sample from the HIV-infected patient;

(b) evaluating whether the plasma sample contains nucleic acid encoding HIV protease having a mutation at codon 88 and additional mutations at codons 63, 77 and/or 46 or a combination thereof; and

20

(c) determining decreased susceptibility to nelfinavir and indinavir and increased susceptibility to amprenavir.

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8. The method of claim 7, wherein the mutation at codon 63 codes for a proline (P) or a glutamine (Q), the mutation at codon 77 codes for an isoleucine (I).and the mutation at codon 46 codes for a leucine (L) or an isoleucine (I).

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9. The method of claim 7, wherein the HIV-infected patient is being treated with an antiretroviral agent.

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10. A method of assessing the effectiveness of protease antiretroviral therapy of an HIV-infected patient comprising:

(a) collecting a plasma sample from the HIV-infected patient;

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(b) evaluating whether the plasma sample contains nucleic acid encoding HIV protease having a mutation at codon 88 and additional mutations at codons 63, 77, 46, 10, 20, and/or 36 or a

- 5 combination thereof; and
- (c) determining decreased susceptibility to nelfinavir and indinavir and increased susceptibility to amprenavir.

10 11. The method of claim 10, wherein the mutation at codon 63 codes for a proline (P) or a glutamine (Q), the mutation at codon 77 codes for an isoleucine (I), the mutation at codon 46 codes for a leucine (L) or an isoleucine (I), the mutation at codon 10 codes for a  
15 isoleucine (I) or a phenylalanine (F), the mutation at 20 codes for a threonine (T) or a methionine (M) or an arginine (R), and the mutation at 36 codes for an isoleucine (I) or a valine (V).

20 12. The method of claim 10, wherein the HIV-infected patient is being treated with an antiretroviral agent.

13. A method for evaluating the biological effectiveness of a candidate HIV antiretroviral drug compound  
25 comprising:

- (a) introducing a resistance test vector comprising a patient-derived segment further comprising a mutation at codon 88 and an indicator gene into a host cell;
- 30 (b) culturing the host cell from step (a);
- (c) measuring the indicator in a target host cell; and
- (d) comparing the measurement of the indicator from step (c) with the measurement of the indicator measured when steps (a) - (c) are carried out in  
35 the absence of the candidate antiretroviral drug compound;

wherein a test concentration of the candidate antiretroviral drug compound is present at steps (a) - (c); at steps (b) - (c); or at step (c).

5        14. A method for evaluating the biological effectiveness of  
a candidate HIV antiretroviral drug compound  
comprising:

10            (a) introducing a resistance test vector comprising a  
patient-derived segment further comprising a  
mutation at codon 88 and mutation(s) at codons 63  
and/or 77 or a combination thereof and an  
indicator gene into a host cell;

(b) culturing the host cell from step (a);

15            (c) measuring the indicator in a target host cell; and

20            (d) comparing the measurement of the indicator from  
step (c) with the measurement of the indicator  
measured when steps (a) - (c) are carried out in  
the absence of the candidate antiretroviral drug  
compound;

wherein a test concentration of the candidate  
antiretroviral drug compound is present at steps (a) -  
(c); at steps (b) - (c); or at step (c).

25        15. A method for evaluating the biological effectiveness of  
a candidate HIV antiretroviral drug compound  
comprising:

30            (a) introducing a resistance test vector comprising a  
patient-derived segment further comprising a  
mutation at codon 88 and mutation(s) at codons 63,  
77, and/or 46 or a combination thereof and an  
indicator gene into a host cell;

(b) culturing the host cell from step (a);

35            (c) measuring the indicator in a target host cell; and

40            (d) comparing the measurement of the indicator from  
step (c) with the measurement of the indicator  
measured when steps (a) - (c) are carried out in  
the absence of the candidate antiretroviral drug  
compound;

5 wherein a test concentration of the candidate antiretroviral  
drug compound is present at steps (a) - (c); at steps (b) -  
(c); or at step (c).

- 10 16. A method for evaluating the biological effectiveness of  
a candidate HIV antiretroviral drug compound comprising:  
(a) introducing a resistance test vector comprising a  
patient-derived segment further comprising a  
mutation at codon 88 and mutation(s) at codons 63,  
77, 46, 10, 20, and/or 36 or a combination thereof  
15 and an indicator gene into a host cell;  
(b) culturing the host cell from step (a);  
(c) measuring the indicator in a target host cell; and  
(d) comparing the measurement of the indicator from  
step (c) with the measurement of the indicator  
20 measured when steps (a) - (c) are carried out in  
the absence of the candidate antiretroviral drug  
compound;

wherein a test concentration of the candidate antiretroviral  
drug compound is present at steps (a) - (c); at steps (b) -  
25 (c); or at step (c).

17. A resistance test vector comprising an HIV —  
patient-derived segment further comprising protease  
having a mutation at codon 88 and an indicator gene,  
30 wherein the expression of the indicator gene is  
dependent upon the patient derived segment.

18. The resistance test vector of claim 17, wherein the  
patient-derived segment having a mutation at codon 88  
35 further comprises mutations at codons 63 and 77 or a  
combination thereof.

19. The resistance test vector of claim 17, wherein the  
patient-derived segment having a mutation at codon 88  
40 further comprises mutations at codons 63, 77 and/or 46

5 or a combination thereof.

20. The resistance test vector of claim 17, wherein the  
patient-derived segment having a mutation at codon 88  
further comprises mutations at codons 63, 77, 46, 10,  
10 20 and/or 36 or a combination thereof.

21. A method for evaluating the viral fitness of a  
patient's virus comprising:

- 15 (a) introducing a resistance test vector comprising a  
patient-derived segment from a patient's virus and  
an indicator gene into a host cell;
- (b) culturing the host cell from step (a);
- (c) measuring the luciferase activity in a target host  
cell in the absence of any antiretroviral drug;  
20 and
- (d) comparing the measurement of the indicator from  
step (c) with the measurement of the indicator  
measured when steps (a)-(c) are carried out for a  
reference control in the absence of any  
25 antiretroviral drug;

wherein a reduction in the luciferase activity measured in  
step (c) as compared to step (d) indicates a reduction in  
viral fitness.

30 22. A method of assessing the effectiveness of protease  
antiretroviral therapy of an HIV-infected patient comprising:

- (a) collecting a plasma sample from the HIV-infected  
patient;
- 35 (b) evaluating whether the plasma sample contains  
nucleic acid encoding HIV protease having a  
mutation at codon 82 and secondary positions; and
- (c) determining changes in susceptibility to  
ritonavir, nelfinavir, indinavir, saquinavir and  
amprenavir.

5        23. The method of claim 22, wherein the mutation at codon  
         82 codes for alanine (A), phenylalanine (F), serine  
         (S), or threonine (T).

10       24. The method of claim 22, wherein the HIV-infected  
         patient is being treated with an antiretroviral agent.

25. A method of assessing the effectiveness of protease  
antiretroviral therapy of an HIV-infected patient  
comprising:

15       (a) collecting a plasma sample from the  
         HIV-infected patient;

20       (b) evaluating whether the plasma sample contains  
         nucleic acid encoding HIV protease having a  
         mutation at codon 82 and an additional  
         mutation at codon 24; and

         (c) determining decreased susceptibility to  
         indinavir.

25       26. The method of claim 25, wherein the mutation at codon  
         24 codes for an isoleucine (I).

27. The method of claim 25, wherein the HIV-infected  
patient is being treated with an antiretroviral agent.

30       28. A method of assessing the effectiveness of protease  
antiretroviral therapy of an HIV-infected patient  
comprising:

         (a) collecting a plasma sample from the HIV-infected  
         patient;

35       (b) evaluating whether the plasma sample contains  
         nucleic acid encoding HIV protease having a  
         mutation at codon 82 and an additional mutation at  
         codon 71; and

         (c) determining decreased susceptibility to indinavir.

- 5        29. The method of claim 28, wherein the mutation at codon  
71 codes for an amino acid selected from the group  
consisting of a threonine, (T) valine, (V) leucine (L)  
and isoleucine (I).
- 10       30. The method of claim 28, wherein the HIV-infected  
patient is being treated with an antiretroviral  
agent.
- 15       31. A method of assessing the effectiveness of protease  
antiretroviral therapy of an HIV-infected patient  
comprising:  
      (a) collecting a plasma sample from the HIV-infected  
          patient;  
      (b) evaluating whether the plasma sample contains  
20        nucleic acid encoding HIV protease having a  
mutation at codon 82 and additional mutations at  
codons selected from the group consisting of codon  
54, 46, 10, 63, and a combination thereof; and  
      (c) determining decreased susceptibility to indinavir.
- 25       32. The method of claim 31, wherein the mutation at codon  
54 codes for an amino acid selected from the group  
consisting of a valine (V), alanine (A), leucine (L)  
and threonine (T), the mutation at codon 46 codes for  
30        an amino acid selected from the group consisting of a  
leucine (L), isoleucine (I) and valine (V), the  
mutation at codon 10 codes for an amino acid selected  
from the group consisting of an isoleucine (I), valine  
(V), phenylalanine (F), and arginine (R), and the  
35        mutation at codon 63 codes for an amino acid selected  
from the group consisting of proline (P), alanine (A),  
serine (S), threonine (T), glutamine(Q), , cysteine  
(C), and valine (V).



- 5        33. The method of claim 31, wherein the HIV-infected  
patient is being treated with an antiretroviral  
agent.
- 10       34. A method of assessing the effectiveness of protease  
antiretroviral therapy of an HIV-infected patient  
comprising:  
      (a) collecting a plasma sample from the HIV-infected  
          patient;  
      (b) evaluating whether the plasma sample contains  
15        nucleic acid encoding HIV protease having a  
          mutation at codon 82 and an additional mutation at  
          codon 20; and  
      (c) determining decreased susceptibility to saquinavir.
- 20       35. The method of claim 34, wherein the mutation at codon  
20 codes for an amino acid selected from the group  
consisting of a methionine (M), threonine (T),  
isoleucine (I), and arginine (R).
- 25       36. The method of claim 34, wherein the HIV-infected  
patient is being treated with an antiretroviral  
agent.
- 30       37. A method of assessing the effectiveness of protease  
antiretroviral therapy of an HIV-infected patient  
comprising:  
      (a) collecting a plasma sample from the HIV-infected  
          patient;  
      (b) evaluating whether the plasma sample contains  
35        nucleic acid encoding HIV protease having a  
          mutation at codon 82 and an additional mutation at  
          codon 36; and  
      (c) determining decreased susceptibility to saquinavir.

- 5        38. The method of claim 37, wherein the mutation at codon  
36 for an amino acid selected from the group consisting  
of a isoleucine (I), leucine (L), and valine (V).
- 10       39. The method of claim 37, wherein the HIV-infected  
patient is being treated with an antiretroviral  
agent.
- 15       40. A method of assessing the effectiveness of protease  
antiretroviral therapy of an HIV-infected patient  
comprising:  
      (a) collecting a plasma sample from the HIV-infected  
          patient;  
      (b) evaluating whether the plasma sample contains  
          nucleic acid encoding HIV protease having a  
20        mutation at codon 82 and additional mutations at  
          codons 24, 71, 54, and/or 10 or a combination  
          thereof; and  
      (c) determining decreased susceptibility to saquinavir.
- 25       41. The method of claim 40, wherein the mutation at codon  
24 codes for an isoleucine (I), the mutation at codon  
71 codes for an amino acid selected from the group  
consisting of a threonine (T), valine (V), leucine (L),  
and isoleucine (I), the mutation at codon 54 codes for  
30       an amino acid selected from the group consisting of  
          valine (V), alanine (A), leucine (L), and threonine  
          (T), and the mutation at codon 10 codes for an amino  
          acid selected from the group consisting of an  
          isoleucine (I), valine (V), phenylalanine (F), and  
35        arginine(R).
- 40       42. The method of claim 40, wherein the HIV-infected  
patient is being treated with an antiretroviral  
agent.

5 43. A method of assessing the effectiveness of protease  
antiretroviral therapy of an HIV-infected patient  
comprising:

- (a) collecting a plasma sample from the HIV-infected  
patient;
- 10 (b) evaluating whether the plasma sample contains  
nucleic acid encoding HIV protease having a  
mutation at codon 82 and the number of additional  
mutations at secondary positions; and
- (c) determining decreased susceptibilty to indinavir  
and saquinavir.
- 15

44. The method of claim 43, wherein the number of  
additional mutations at secondary positions is at least  
3.

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45. A method of assessing the effectiveness of protease  
antiretroviral therapy of an HIV-infected patient  
comprising:

- (ss) collecting a plasma sample from the HIV-infected  
patient;
- 25 (b) evaluating whether the plasma sample contains  
nucleic acid encoding HIV protease having a  
mutation at codon 90 and secondary mutations; and
- (c) determining changes in susceptibility to  
ritonavir, nelfinavir, indinavir, saquinivir and  
amprenavir.
- 30

46. The method of claim 45, wherein the mutation at codon  
90 codes for a methionine.

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47. The method of claim 45, wherein the HIV-infected  
patient is being treated with an antiretroviral  
agent.

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- 5        48. A method of assessing the effectiveness of protease  
antiretroviral therapy of an HIV-infected patient  
comprising:  
      (d) collecting a plasma sample from the HIV-infected  
          patient;  
10        (b) evaluating whether the plasma sample contains  
nucleic acid encoding HIV protease having a  
mutation at codon 90 and an additional mutation at  
codon 73; and  
      (c) determining decreased susceptibilty to indinavir.  
15        49. The method of claim 48, wherein the mutation at codon  
73 codes for an amino acid selected from the group  
consisting of a serine (S), threonine (T), and cysteine  
(C).
- 20        50. The method of claim 48, wherein the HIV-infected  
patient is being treated with an antiretroviral  
agent.
- 25        51. A method of assessing the effectiveness of protease  
antiretroviral therapy of an HIV-infected patient  
comprising:  
      (d) collecting a plasma sample from the HIV-infected  
          patient;  
30        (b) evaluating whether the plasma sample contains  
nucleic acid encoding HIV protease having a  
mutation at codon 90 and an additional mutation at  
codon 71; and  
      (c) determining decreased susceptibilty to indinavir.  
35        52. The method of claim 51, wherein the mutation at codon  
71 codes for an amino acid selected from the group  
consisting of a threonine (T), valine (V), leucine (L),  
and isoleucine (I).

5 53. The method of claim 51, wherein the HIV-infected patient is being treated with an antiretroviral agent.

54. A method of assessing the effectiveness of protease antiretroviral therapy of an HIV-infected patient comprising:

- 10 (d) collecting a plasma sample from the HIV-infected patient;
- (b) evaluating whether the plasma sample contains nucleic acid encoding HIV protease having a mutation at codon 90 and an additional mutation at
- 15 codon 46,; and
- (c) determining decreased susceptibilty to indinavir.

55. The method of claim 54, wherein the mutation at codon 46 codes for an amino acid selected from the group consisting of a leucine (L), isoleucine (I) and valine (V).

56. The method of claim 54, wherein the HIV-infected patient is being treated with an antiretroviral agent.

57. A method of assessing the effectiveness of protease antiretroviral therapy of an HIV-infected patient comprising:

- 30 (d) collecting a plasma sample from the HIV-infected patient;
- (b) evaluating whether the plasma sample contains nucleic acid encoding HIV protease having a mutation at codon 90 and an additional mutation at
- 35 codon 73; and
- (c) determining decreased susceptibilty to saquinavir.

58. The method of claim 57, wherein the mutation at codon 73 codes for an amino acid selected from the group

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5 consisting of a serine (S), threonine (T), and cysteine  
(C).

10 59. The method of claim 57, wherein the HIV-infected  
patient is being treated with an antiretroviral  
agent.

60. A method of assessing the effectiveness of protease  
antiretroviral therapy of an HIV-infected patient  
comprising:

15 (d) collecting a plasma sample from the HIV-infected  
patient;

(b) evaluating whether the plasma sample contains  
nucleic acid encoding HIV protease having a  
mutation at codon 90 and an additional mutation at  
20 codon 71; and

(c) determining decreased susceptibility to saquinavir.

25 61. The method of claim 60, wherein the mutation at codon  
71 codes for an amino acid selected from the group  
consisting of a threonine (T), valine (V), leucine (L),  
and isoleucine (I).

30 62. The method of claim 60, wherein the HIV-infected  
patient is being treated with an antiretroviral  
agent.

63. A method of assessing the effectiveness of protease  
antiretroviral therapy of an HIV-infected patient  
comprising:

35 (d) collecting a plasma sample from the HIV-infected  
patient;

(b) evaluating whether the plasma sample contains  
nucleic acid encoding HIV protease having a  
mutation at codon 90 and additional mutations at  
40 codons 77 and 10; and

5 (c) determining decreased susceptibility to saquinavir.

64. The method of claim 63, wherein the mutation at codon  
77 codes for an amino acid selected from the group  
consisting of isoleucine (I) and threonine (T) and the  
10 mutation at codon 10 codes for an amino acid selected  
from the group consisting of isoleucine (I), valine  
(V), phenylalanine (F), and arginine (R).

65. The method of claim 63, wherein the HIV-infected  
15 patient is being treated with an antiretroviral  
agent.

66. A method of assessing the effectiveness of protease  
antiretroviral therapy of an HIV-infected patient  
20 comprising:

- (d) collecting a plasma sample from the HIV-infected  
patient;
- (b) evaluating whether the plasma sample contains  
nucleic acid encoding HIV protease having a  
25 mutation at codon 90 and the number of additional  
mutations at secondary positions; and
- (c) determining decreased susceptibility to indinavir  
and saquinavir.

67. The method of claim 66, wherein the number of additional  
30 mutations at secondary positions is at least 3.

68. A method of assessing the effectiveness of protease  
antiretroviral therapy of an HIV-infected patient  
35 comprising:

- (a) collecting a plasma sample from the HIV-infected  
patient;
- (b) evaluating whether the plasma sample contains  
nucleic acid encoding HIV protease having a mutation at  
40 codons 82 and 90 and secondary mutations; and

(c) determining changes in susceptibility to ritonavir, nelfinavir, indinavir, saquinavir and amprenavir.

69. The method of claim 68, wherein the mutation at codon 82 codes for an amino acid selected from the group consisting of alanine (A), phenylalanine (F), serine (S), and threonine (T) and the mutation at codon 90 codes for a methionine (M).

70. The method of claim 68, wherein the HIV-infected patient is being treated with an antiretroviral agent.

71. A method for evaluating the biological effectiveness of a candidate HIV protease antiretroviral drug compound comprising:

(a) introducing a resistance test vector comprising a patient-derived segment further comprising a mutation at codon 82 and additional mutations at one or more secondary positions and an indicator gene into a host cell;

(b) culturing the host cell from step (a);

(c) measuring the indicator in a target host cell; and

(d) comparing the measurement of the indicator from step (c) with the measurement of the indicator measured when steps (a) - (c) are carried out in the absence of the candidate antiretroviral drug compound;

wherein a test concentration of the candidate antiretroviral drug compound is present at steps (a) - (c); at steps (b) - (c); or at step (c).



5 72. A method for evaluating the biological effectiveness of  
a candidate HIV protease antiretroviral drug compound  
comprising:

- 10 (a) introducing a resistance test vector comprising a  
patient-derived segment further comprising a  
mutation at codon 82 and secondary mutation(s) at  
codons 20, 24, 71, 54 and/or 10 or a combination  
thereof and an indicator gene into a host cell;  
(b) culturing the host cell from step (a);  
(c) measuring the indicator in a target host cell; and  
15 (d) comparing the measurement of the indicator from  
step (c) with the measurement of the indicator  
measured when steps (a) - (c) are carried out in  
the absence of the candidate antiretroviral drug  
compound;

20 wherein a test concentration of the candidate  
antiretroviral drug compound is present at steps (a) -  
(c); at steps (b) - (c); or at step (c).

25 73. A method for evaluating the biological effectiveness of  
a candidate HIV protease antiretroviral drug compound  
comprising:

- 30 (a) introducing a resistance test vector comprising a  
patient-derived segment further comprising a  
mutation at codon 90 and additional mutations at  
one or more secondary positions and an indicator  
gene into a host cell;  
(b) culturing the host cell from step (a);  
(c) measuring the indicator in a target host cell; and  
(d) comparing the measurement of the indicator from  
35 step (c) with the measurement of the indicator  
measured when steps (a) - (c) are carried out in  
the absence of the candidate antiretroviral drug  
compound;

5            wherein a test concentration of the candidate  
antiretroviral drug compound is present at steps (a) -  
(c); at steps (b) - (c); or at step (c).

74. A method for evaluating the biological effectiveness of  
10 a candidate HIV protease antiretroviral drug compound  
comprising:

- 15            (a) introducing a resistance test vector comprising a  
patient-derived segment further comprising a  
mutation at codon 90 and secondary mutation(s) at  
codons 73, 71, 10 and/or 46 or a combination  
thereof and an indicator gene into a host cell;  
             (b) culturing the host cell from step (a);  
             (c) measuring the indicator in a target host cell; and  
             (d) comparing the measurement of the indicator from  
20 step (c) with the measurement of the indicator  
measured when steps (a) - (c) are carried out in  
the absence of the candidate antiretroviral drug  
compound;

25            wherein a test concentration of the candidate  
antiretroviral drug compound is present at steps (a) -  
(c); at steps (b) - (c); or at step (c).

75. A method for evaluating the biological effectiveness of  
30 a candidate HIV protease antiretroviral drug compound  
comprising:

- 35            (a) introducing a resistance test vector comprising a  
patient-derived segment further comprising a  
mutation at codons 82 and 90 and additional  
mutations at one or more secondary positions and  
an indicator gene into a host cell;  
             (b) culturing the host cell from step (a);  
             (c) measuring the indicator in a target host cell; and  
             (d) comparing the measurement of the indicator from  
40 step (c) with the measurement of the indicator

5 measured when steps (a) - (c) are carried out in  
the absence of the candidate antiretroviral drug  
compound;

10 wherein a test concentration of the candidate  
antiretroviral drug compound is present at steps (a) -  
(c); at steps (b) - (c); or at step (c).

15 76. A resistance test vector comprising an HIV patient-  
derived segment further comprising protease having a  
mutation at codon 82 and an indicator gene, wherein the  
expression of the indicator gene is dependent upon the  
patient derived segment.

20 77. The resistance test vector of claim 76, wherein the  
patient-derived segment having a mutation at codon 82  
further comprises at least one secondary mutation at  
a codon selected from the group consisting of 20, 24,  
71, 54, 10 and a combination thereof.

25 78. The resistance test vector of claim 76, wherein the  
patient-derived segment having a mutation at codon 90  
further comprises at least one secondary mutation at a  
codon selected from the group consisting of 73, 71, 46,  
10 and a combination thereof.

30 79. A method for determining replication capacity for a  
patient's virus comprising:

- 35 (a) introducing a resistance test vector comprising a  
patient derived segment and an indicator gene into  
a host cell;
- (b) culturing the host cell from (a);
- (c) harvesting viral particles from step (b) and  
infecting target host cells;
- 40 (d) measuring expression of the indicator gene in the  
target host cell, wherein the expression of the

5 indicator gene is dependent upon the patient-derived segment;

- 10 (e) comparing the expression of the indicator gene from (d) with the expression of the indicator gene measured when steps (a) through (d) are carried out in a control resistance test vector; and
- (f) normalizing the expression of the indicator gene by measuring an amount of virus in step (c).

15 80. A method of assessing the effectiveness of protease antiretroviral therapy of an HIV-infected patient comprising:

- 20 (a) collecting a biological sample from the HIV-infected patient;
- (b) evaluating whether the biological sample contains nucleic acid encoding HIV protease having a mutation at codon 82 or codon 90; and
- (c) determining changes in susceptibility to protease inhibitors.

25 81. The method of claim 80, wherein step (c) determines changes in susceptibility to saquinavir.

30 82. The method of claim 80, wherein the mutation at codon 82 codes for alanine (A), phenylalanine (F), serine (S), or threonine (T).

35 83. The method of claim 82, wherein the mutation at codon 82 is a substitution of alanine (A), phenylalanine (F), serine (S), or threonine (T) for valine (V).

84. The method of claim 80, wherein the mutation at codon 90 codes for methionine (M).

40 85. The method of claim 84, wherein the mutation at codon 90 is a substitution of methionine (M) for leucine (L).

86. A method for evaluating the biological effectiveness of a candidate HIV protease antiretroviral drug compound comprising:

(a) introducing a resistance test vector comprising a patient-derived segment having nucleic acid encoding HIV protease with a mutation at codon 82 or codon 90 and an indicator gene into a host cell;

(b) culturing the host cell from step (a);

(c) measuring the indicator gene in a target host cell; and

(d) comparing the measurement of the indicator gene from step (c) with the measurement of the indicator gene measured when steps (a) - (c) are carried out in the absence of the candidate antiretroviral drug compound;

wherein a test concentration of the candidate antiretroviral drug compound is present at steps (a) - (c); at steps (b) - (c); or at step (c).

87. A resistance test vector comprising an HIV patient-derived segment further comprising protease having a mutation at codon 82 or codon 90 and an indicator gene, wherein the expression of the indicator gene is dependent upon the patient-derived segment.

88. The resistance test vector of claim 87, wherein the patient-derived segment having a mutation at codon 82 codes for alanine (A), phenylalanine (F), serine (S), or threonine (T).

89. The resistance test vector of claim 88, wherein the patient-derived segment having a mutation at codon 82 is a substitution of alanine (A), phenylalanine (F), serine (S), or threonine (T) for valine(V).

5 90. The resistance test vector of claim 87, wherein the patient-derived segment having a mutation at codon 90 codes for methionine (M).

10 91. The resistance test vector of claim 90, wherein the patient-derived segment having a mutation at codon 90 is a substitution of methionine (M) for leucine (L).

15 92. A method for determining replication capacity for a patient's virus comprising:

- 20 (a) introducing a resistance test vector comprising a patient-derived segment and an indicator gene into a host cell;
- (b) culturing the host cell from (a);
- (c) harvesting viral particles from step (b) and infecting target host cells;
- 25 (d) measuring expression of the indicator gene in the target host cell, wherein the expression of the indicator gene is dependent upon the patient-derived segment; and
- (e) comparing the expression of the indicator gene from (d) with the expression of the indicator gene measured when steps (a) through (d) are carried out in a control resistance test vector.

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93. The method of claim 92 further comprising the step of:

- (f) normalizing the expression of the indicator gene by measuring an amount of virus in step (c).

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94. The method of claim 92 wherein the patient-derived segment comprises nucleic acid encoding HIV integrase having a mutation at codon 66.

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5 95. The method of claim 92 wherein the patient-derived  
segment comprises nucleic acid encoding HIV integrase  
having a mutation at codon 154.

10 96. The method of claim 94 wherein the patient-derived  
segment comprises nucleic acid encoding HIV integrase  
having an additional mutation at codon 153.

15 97. The method of claim 94 wherein the patient-derived  
segment comprises nucleic acid encoding HIV integrase  
having an additional mutation at codon 154.

20 98. A method of assessing the effectiveness of protease  
antiretroviral therapy of an HIV-infected patient  
comprising:

(uuuu) collecting a biological sample from the  
HIV-infected patient;

25 (b) evaluating whether the biological sample contains  
nucleic acid encoding HIV protease having a  
mutation at codon 82 and a secondary mutation at  
codons selected from the group consisting of 73,  
55, 48, 20, 43, 53, 90, 13, 84, 23, 33, 74, 32,  
39, 60, 36, and 35, or a mutation at codon 90 and  
a secondary mutation at codons selected from the  
group consisting of 53, 95, 54, 84, 82, 46, 13,  
30 74, 55, 85, 20, 72, 62, 66, 84, 48, 33, 73, 71,  
64, 93, 23, 58, and 36; and

(c) determining a change in susceptibility to a  
protease inhibitor.

35 99. The method of claim 98, wherein the mutation at codon  
82 is a substitution of alanine (A), phenylalanine (F),  
serine (S), or threonine (T) for valine(V) and the  
mutation at codon 90 is a substitution of methionine (M)  
for leucine (L).

- 5 100. The method of claim 99, wherein the protease inhibitor  
is selected from the group consisting of indinavir,  
amprenavir, and saquinavir.
- 10 101. The method of claim 100, having a mutation at codon 82  
and a secondary mutation at codons selected from the  
group consisting of 84, 48, 23, 73, 53, 33, 74, 20, 90,  
32 and 39 or a mutation at codon 90 and a secondary  
15 mutation at codons selected from the group consisting  
of 53, 66, 84, 54, 48, 33, 73, 20, 71, 64 and 93,  
wherein the protease inhibitor is saquinavir.
- 20 102. The method of claim 101, having a mutation at codon 82  
and a secondary mutation at codons selected from the  
group consisting of 84, 48, 23, 73, 53, 33, 74, 20, and  
90, or a mutation at codon 90 and a secondary mutation  
at codons selected from the group consisting of 53, 66,  
84, 54, 48, 33, 73, 20, and 71, wherein the change in  
susceptibility in step (c) is a decrease in  
25 susceptibility to saquinavir.
- 30 103. The method of claim 101, having a mutation at codon 82  
and a secondary mutation at codons 32 or 39, or a  
mutation at codon 90 and a secondary mutation at codons  
64 or 93, wherein the change in susceptibility in step  
(c) is an increase in susceptibility to saquinavir.
- 35 104. The method of claim 100, having a mutation at codon 90  
and a secondary mutation at codons selected from the  
group consisting of 53, 95, 54, 84, 82, 46, 13, and 74,  
wherein the protease inhibitor is indinavir.
- 40 105. The method of claim 104, having a mutation at codon 90  
and a secondary mutation at codons selected from the  
group consisting of 53, 95, 54, 84, 82, and 46, wherein  
the change in susceptibility in step (c) is a decrease



5 in susceptibility to indinavir.

106. The method of claim 104, having a mutation at codon 90 and a secondary mutation at codons 13 or 74, wherein the change in susceptibility in step (c) is an increase in  
10 susceptibility to indinavir.

107. The method of claim 100, having a mutation at codon 82 and a secondary mutation at codons selected from the group consisting of 73, 55, 48, 20, 43, 53, 90, 13, 48,  
15 23, 84, 53, 74, 60, 33, 36, 35, 32, and 46 or a mutation at codon 90 and a secondary mutation at codons selected from the group consisting of 95, 55, 54, 82, 85, 84, 20, 72, 62, 74, 53, 48, 23, 58, 36, 64, 77, and 93.

108. The method of claim 107, wherein the protease inhibitor is selected from the group consisting of indinavir, amprenavir, and saquinavir.

109. The method of claim 108, wherein step (c) is determining  
25 a change in susceptibility to the protease inhibitor greater than 10 fold.

110. The method of claim 108, having a mutation at codon 82 and a secondary mutation at codons selected from the group consisting of 48, 23, 84, 53, 74, 20, 60, 33, 36,  
30 35, or a mutation at codon 90 and a secondary mutation at codons selected from the group consisting of 84, 53, 48, 23, 58, 20, 36, and 54, wherein the change in susceptibility in step (c) is a decrease in  
35 susceptibility to saquinavir.

111. The method of claim 108, having a mutation at codon 82 and a secondary mutation at codons 32 or 46, or a mutation at codon 90 and a secondary mutation at codons  
40 64, 77, or 93, wherein the change in susceptibility in

5           step (c) is an increase in susceptibility to saquinavir.

112. The method of claim 108, having a mutation at codon 82  
and a secondary mutation at codons selected from the  
group consisting of 73, 55, 48, 20, 43, 53, and 90, or  
10       a mutation at codon 90 and a secondary mutation at  
codons selected from the group consisting of 95, 55, 54,  
82, 85, 84, 20, 72, and 62, wherein the change in  
susceptibility in step (c) is a decrease in  
susceptibility to indinavir.

15           113. The method of claim 108, having a mutation at codon 82  
and a secondary mutation at codon 13, or a mutation at  
codon 90 and a secondary mutation at codon 74, wherein  
the change in susceptibility in step (c) is an increase  
20       in susceptibility to indinavir.

114. A method of assessing the effectiveness of protease  
antiretroviral therapy of an HIV-infected patient  
comprising:

25       (kkkkk)   collecting a biological sample from the  
                  HIV-infected patient;

(b)   evaluating whether the biological sample contains  
nucleic acid encoding HIV protease having a  
mutation at codon 90 and secondary mutations of at  
30       least three codons; and

(c)   determining a decrease in susceptibility to  
saquinavir.

115. The method of claim 114, wherein in the evaluating step  
35       (b), the nucleic acid encoding HIV protease has  
secondary mutations of at least five codons.

116. The method of claim 114, wherein the secondary mutation  
are selected from the group consisting of codons 10, 20,  
40       52, 53, 54, 66, 71, 73 and 84.

5 117. A method of assessing the effectiveness of protease  
antiretroviral therapy of an HIV-infected patient  
comprising:

(nnnnn) collecting a biological sample from the  
HIV-infected patient;

10 (b) evaluating whether the biological sample contains  
nucleic acid encoding HIV protease having a  
mutation at codon 82 and secondary mutations at  
codons selected from the group consisting of 33,  
23, 84, 32, 53, 90, 37, 71, 46, 10, 54, 61, 11,  
15 and 46, or a mutation at codon 90 and secondary  
mutations at codons selected from the group  
consisting of 89, 53, 84, 33, 92, 95, 54, 58, 46,  
82, 36, 10, 62, 74, 15, 47, 66, 32, 55, 53, 13,  
and 69; and

20 (c) determining a change in susceptibility to  
amprenavir.

118. The method of claim 117, wherein the mutation at codon  
82 is a substitution of alanine (A), phenylalanine (F),  
25 serine (S), or threonine (T) for valine (V) and the  
mutation at codon 90 is a substitution of methionine (M)  
for leucine (L).

119. The method of claim 118, having a mutation at codon 82  
30 and secondary mutations at codons selected from the  
group consisting of 33, 23, 84, 32, 53, 90, 37, 71, 46,  
10, 54, 11, and 46, or a mutation at codon 90 and  
secondary mutations at codons selected from the group  
consisting of 89, 53, 84, 33, 92, 95, 54, 58, 46, 82,  
35 36, 10, 62, 47, 66, 32, 55, 53, and 13; wherein the  
change in susceptibility in step (c) is a decrease in  
susceptibility to saquinavir.

120. The method of claim 118, having a mutation at codon 82  
40 and a secondary mutation at codon 61, or a mutation at

5 codon 90 and secondary mutations at codons 74, 15, or  
69, wherein the change in susceptibility in step (c) is  
an increase in susceptibility to saquinavir.

10 121. A resistance test vector comprising an HIV patient-  
derived segment comprising nucleic acid encoding  
protease having a mutation at codon 82 and secondary  
mutations at codons selected from the group consisting  
of 73, 55, 48, 20, 43, 53, 90, 13, 84, 23, 33, 74, 32,  
15 39, 60, 36, and 35, or a mutation at codon 90 and  
secondary mutations at codons selected from the group  
consisting of 53, 95, 54, 84, 82, 46, 13, 74, 55, 85,  
20 20, 72, 62, 66, 84, 48, 33, 73, 71, 64, 93, 23, 58, and  
36 and an indicator gene, wherein the expression of the  
indicator gene is dependent upon the patient-derived  
segment.

25 122. The resistance test vector of claim 121, wherein the  
mutation of the patient derived segment at codon 82 is  
a substitution of alanine (A), phenylalanine (F), serine  
(S), or threonine (T) for valine(V) and the mutation at  
30 codon 90 is a substitution of methionine (M) for leucine  
(L).

35 123. A method for determining whether an HIV virus obtained  
from a patient infected with HIV is resistant to IDV,  
LPV, NFV and RTV which comprises determining whether a  
mutation at position 30 from D to N exists in the HIV  
40 protease obtained from the patient, wherein the presence  
o the mutation indicates that the virus is resistant to  
IDV, LPV, NFV and RTV.

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124. A method for determining whether an HIV virus obtained from a patient infected with HIV is resistant to IDV, LPV, NFV or RTV which comprises determining whether the virus is resistant to any one of IDV, LPV, NFV or RTV, wherein a determination that the virus is resistant to any one of IDV, LPV, NFV or RTV is indicative of the virus being resistant to IDV, LPV, NFV and RTV.

125. A method for determining cross resistance of an HIV virus to RTV and SQV which comprises determining (i) whether position 30 of the HIV protease is D, and (ii) whether the virus is resistant to NFV, wherein a mutation from D to N at position 30 of HIV protease and resistance of the virus to NFV are indicative of cross resistance to IDV and SQV.

126. A method for determining whether an HIV virus obtained from a patient infected with HIV is resistant to LPV and IND which comprises determining whether position 50 of the HIV protease of the virus is I or V, wherein the determination that position 50 is V is indicative of the virus being resistant to LPV and IND.

TABLE A

# Summary of Replication Capacity (RC) and Enzyme Function Results

	LOW RC (<25% of Ref.*)	MEDIUM RC (26-75% of Ref.)	HIGH RC (>75% of Ref.)
% of Total Tested	41% (55)	45% (59)	14% (19)
PR Processing Defects (%p41>10%)	71% (39)	24% (14)	10% (2)
Impaired RT Activity (<25% of reference)	14% (7)	2% (1)	0%
>10 mutations in Protease	62% (34)	22% (13)	5% (1)
>10X reduced susceptibility to NFV	63% (35)	32% (19)	16% (3)

\*Reference virus: NL4-3

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